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Supercritical Fluid Chromatography

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Introduction

There is tremendous analytical interest in polycyclic aromatic compounds (PAC) because of their carcinogenic properties and their impact on the environment. PAC mixtures can be very complex and extend over a wide range of molecular masses. They mainly originate from the combustion of fossil fuels and are widespread in the environment.

The most commonly used separation techniques for PAC are gas chromatography (GC), high performance liquid chromatography (HPLC) and supercritical fluid chromatography (SFC). GC is often the technique of choice since it works well for low molecular weight and volatile compounds. It is also preferred because the high diffusivity of solutes in gases results in relatively short analysis times. HPLC employs liquids of high solvating power, high densities and high viscosities but is often time-consuming and has much less resolving power than GC.

Separation of PAC by SFC

Although HPLC and GC are commonly used for the analysis of PAC mixtures, SFC has a number of advantages. SCF have diffusivities that are more gas-like, viscosities lower than liquids and densities that are more liquid-like. The resulting mass transfer coefficients lead to a more rapid analysis in SFC than in HPLC. The diffusion coefficients and viscosities of SCF allow GC-like separations on capillary columns but at much lower temperatures because of the high solvating power of SCF. The approach in SFC involves the use of columns of the packed LC type, packed capillary (micro-packed) and the open tubular (capillary) GC type columns.

SFC on Packed Columns

Packed columns usually of the commercial HPLC variety were used in nearly all the early work in SFC. The length and internal diameter (i.d.) of SFC packed columns are constrained by (a) the larger pressure drops as compared to open tubular columns and (b) high flow rates, making interfacing to GC detectors more difficult. Often, narrow-bore packed columns with a diameter of 1 to 2 mm are used because they can be installed in a capillary SFC instrument and are compatible with many detectors.

Early work on the separation of polycyclic aromatic hydrocarbon (PAH) standards revealed that reduced particle size led to increased resolution, and that analysis times were reduced in comparison with HPLC; it was also shown that PAH elution order could be varied by changing the operating temperature and/or the pressure. Marked changes in retention and selectivity are brought about by the addition of a modifier to the mobile phase. Detailed studies of the retention characteristics of PAH on a wide range of packed columns showed that retention in SFC with CO_2-based mobile phases correlates most closely with reverse phase HPLC. Very rapid analysis (< 6 minutes) of the sixteen Environmental Protection Agency target PAH was demonstrated on a 15 cm long column packed with a specially bonded C_{18} silica (**Figure 1**). Efficient separation of PAH can be achieved by coupling columns of different selectivity. Plate numbers up to 220,000 and separation of up to

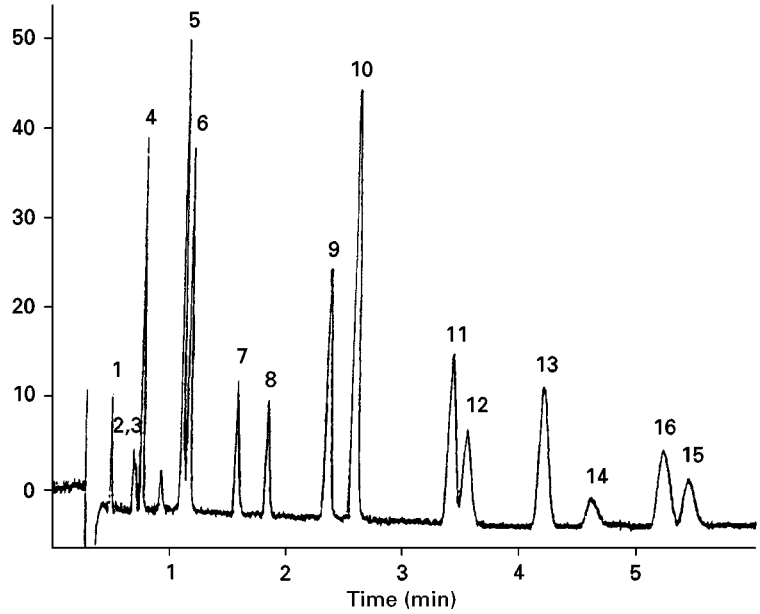

Figure 1 Supercritical fluid chromatography (SFC) of the EPA/PAH mixture. Chromatographic conditions: 5 mL min^{-1}; CO_2/MeOH mobile phase; gradient of 10% MeOH/min from 1% up to 50% then hold at 50%; pressure gradient of 95 bar min^{-1} from 80 bar up to 200 bar then hold at 200 bar; temperature 40°C for 3 minutes then gradient of 7°C per minute to 60°C. Detection UV absorbance at 254 nm. Peak identification 1 naphthalene; 2 acenaphthylene; 3 acenaphthene; 4 fluorene; 5 phenanthrene; 6 anthracene; 7 fluoranthene; 8 pyrene; 9 benz[*a*]anthracene; 10 chrysene; 11 benzo[*b*]fluoranthene; 12 benzo[*k*]fluoranthene; 13 benzo[*a*]pyrene; 14 dibenz[*a*, *h*]anthracene; 15 benzo[*ghi*]perylene; 16 indeno[1,2,3-*cd*']pyrene. Reproduced with permission from Heaton *et al.* (1994).

60 PAH can be achieved by coupling up to seven packed (HPLC) columns together.

SFC on Capillary Columns

Capillary column SFC is often preferred to packed column SFC for separation of PAC mixtures because of better resolution (**Figure 2**). The price paid, however, is much slower analysis and the use of unmodified CO_2 as mobile phase, in which the higher molecular weight PAC are less soluble.

A variety of mobile phases other than CO_2 have been studied in order to increase the selectivity for a wide range of samples and solutes in work which also indicated that the choice of stationary phase for capillary SFC must take into account the solvating power and the reactivity of the supercritical fluid; the stationary phase must be sufficiently immobilized and must not react with the mobile phase to avoid being washed out of the column by the carrier fluid. Supercritical ammonia has been used because of its high polarity and reasonable critical parameters ($T_c = 132.5$°C, $P_c = 112.5$ atm).

A similar range of stationary phases has been used in the capillary SFC of PAC as has been applied in capillary GC: 100% methyl and 5% phenyl siloxanes, and the more selective 5% biphenyl and cyanopropyl siloxanes. Very selective SFC separations have been shown on liquid crystalline phases (**Figure 3**). Isomers may be resolved on the basis of the length-to-breadth ratio and planarity of PAH since they are compatible with the ordered 'rod-like' structure of the phase.

Detection in SFC of PAC

Flame-ionization (FID) is the most commonly used detector in capillary SFC of PAC, but the UV absorption detector has most often been applied in packed column work because of the presence of modifiers. Photodiode array detection then allows identification of parent PAH. Nitro PAC have been determined by capillary SFC with the use of the thermionic detector (TID); no modification is necessary and the detector is sensitive and stable during density programming. Low concentrations of sulfur-containing PAC have been detected with the aid of the flame photometric detector in capillary SFC. The photoionization detector uses a high-energy UV lamp to ionize the eluted PAH, and offers high sensitivity with some selectivity over non-aromatic compounds.

Spectroscopic detection has often been used in SFC to allow PAC identification. PAC are weak IR absorbers, but may be identified from the FTIR spectra of compact spots deposited by CO_2 mobile phase elimination at the restrictor exit; PAH isomers may be differentiated by their FTIR spectra, e.g. chrysene from

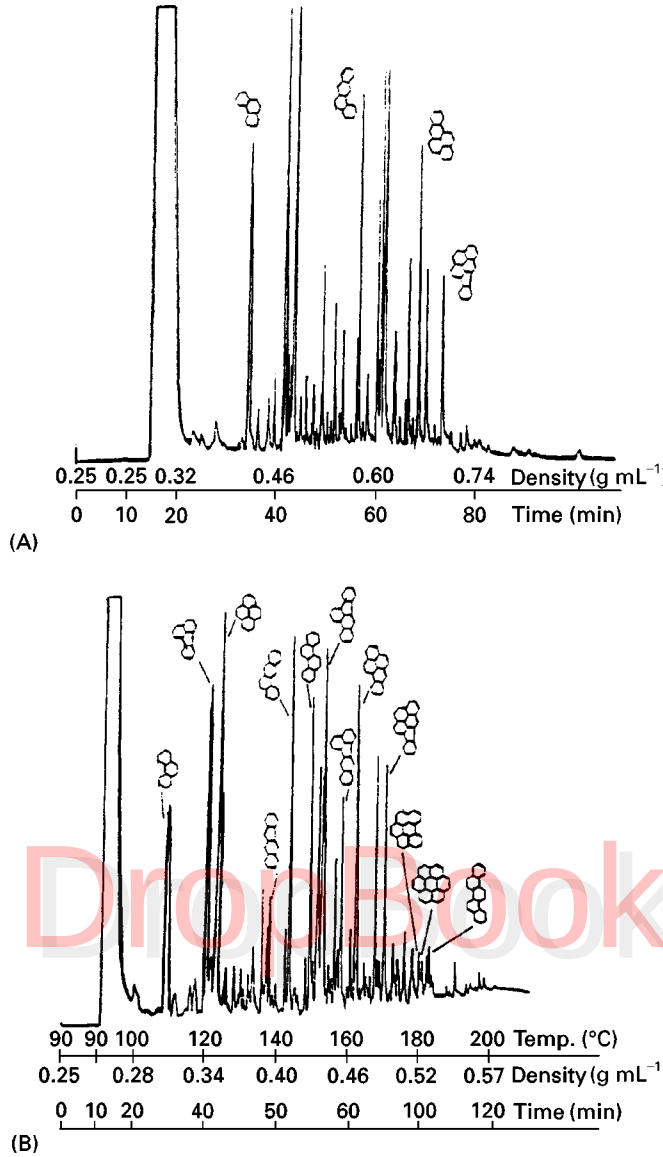

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Figure 2 SFC chromatograms of total coal tar polycyclic aromatic hydrocarbons (A) constant temperature 110°C, density program, (B) simultaneous temperature/density program. Chromatographic conditions: (A) constant temperature at 90°C; density ramp rate of 0.007 g mL^{-1} min^{-1} up to 0.75 g mL^{-1}; 10-m × 0.50-μm i.d fused silica capillary column coated with biphenyl carboxylate ester liquid crystalline (SB-Smectic); FID at 350°C. Reproduced with permission from Kithinji *et al.* (1990) *Journal of High Resolution Chromatography* 13: 27.

benz[*a*]anthracene. Flow-cell FTIR is less useful in SFC of PAC because of the interference from bands from CO_2 mobile phase. Supercritical xenon is a better solvent than CO_2 for PAH, and does not obscure their FTIR spectra but is extremely expensive.

SFC has been interfaced to mass spectrometry (MS) in a number of analyses of PAC; low flow rates in capillary SFC make it particularly useful. SFC-MS is then employed in the identification of PAC especially from their molecular weights. The fluorescence detector has been used in SFC of PAH mixtures with pentane mobile phase. Both excitation and emission spectra have been used in high sensitivity analysis of PAH.

Multidimensional Chromatography

PAC mixtures are often too complex for their analysis in a single chromatographic system. Multidimensional chromatography, generating much higher resolution, and improved separation of PAC mixtures, has been demonstrated in coupled SFC-SFC of both

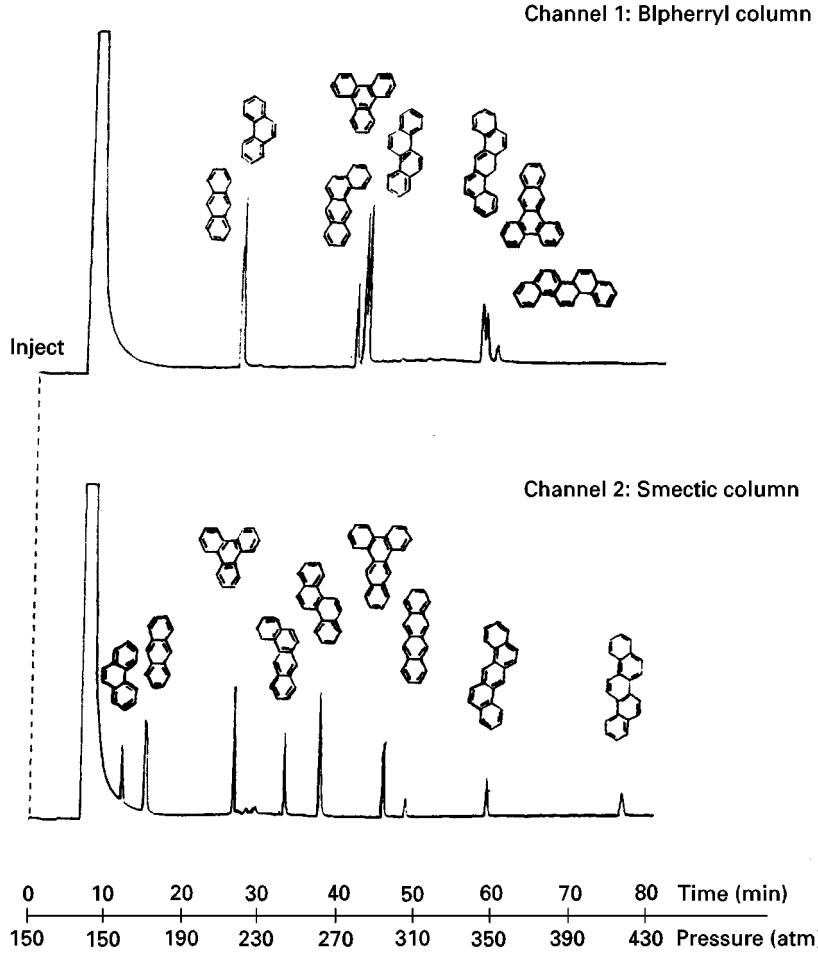
Channel 1: Biphenyl column
Inject
Channel 2: Smectic column
0 10 20 30 40 50 60 70 80 Time (min)
150 150 190 230 270 310 350 390 430 Pressure (atm)

Figure 3 Simultaneous separation of a mixture of 3-, 4- and 5- ring polycyclic aromatic hydrocarbon isomers on biphenyl and smectic columns. Chromatographic conditions: Column 1 – open tubular column coated with a 0.25-μm film of 30% biphenyl-methyl-polysiloxane stationary phase (Biphenyl-30); Column 2 – open tubular column coated with a 0.25-μm film of smectic biphenylcarboxylate ester liquid crystalline polysiloxane stationary phase (SB-Smectic-50); CO_2 at 120°C; pressure program from 150 atm (hold for 10 min) to 450 atm min^{-1}; FID at 300°C. Reproduced with permission from Raynor *et al.* (1990) *Journal of High Resolution Chromatography* 13: 22.

packed column–capillary column and capillary column–capillary column arrangements. The first column can provide chemical class separation, while the second resolves closely related compounds and isomers.

Applications of SFC to PAC Mixtures

SFC is a useful procedure in PAC analysis by providing complementary information to that from GC and HPLC.

Group-type separation by HPLC and open-column chromatograph is unsatisfactory, and SFC methods have proven markedly superior. Packed columns (often silica and silver-salt impregnated silica in tandem) were first used for this application, but certain separations (e.g. saturates from olefins) were often inadequate unless supercritical SF_6 or complex column switching were employed.

Aromatic content of fuels is determined by SFC in an ASTM method on a silica packed column with CO_2. Separation of aromatic compounds on the basis of ring number has been demonstrated on packed capillary columns, and also by two-dimensional capillary SFC–SFC. FID detection with CO_2 mobile phase allows ready quantitation.

Simulated distillation (SIMDIS) of fuels is often used to replace actual distillation in the determination of boiling point distribution. While GC is often used in SIMDIS, damage to columns and sample degradation resulting from the high temperatures necessary to elute high MW compounds has led to the development of SFC methods in which oven temperatures as low as 150°C are sufficient to elute PAC boiling beyond 750°C because of their greater solubility in supercritical CO_2 mobile phase at densities up to 0.71 g mL^{-1}. A column packed with

C_6 bonded silica was especially useful in correlating SFC retention with boiling point for a variety of PAC types.

PAC from fossil fuel and environmental samples have commonly been analysed by SFC, with an application range beyond that of GC and much improved resolution over HPLC methods. Coal-derived PAH containing as many as twelve rings have been eluted in SFC. Identification and quantitation is straightforward with the aid of FID, UV and MS detectors. Very rapid analysis of environmental mixtures of PAC has been demonstrated on a C_{18} column with CO_2/methanol mobile phase and simultaneous pressure, temperature and composition programming.

Many nitrogen-containing PAC cannot be analysed by GC because they are thermally labile, and SFC with TID has been especially useful in allowing identification of hydroxynitrofluorenes and nitropyrenequinones.

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Thin-Layer (Planar) Chromatography

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Polycyclic aromatic hydrocarbons (PAH) originate from organic materials (i.e. oil, gasoline, tobacco, garbage, etc.) due to pyrolysis or incomplete combustion. Several hundred different PAH are known, and traces of them can be found nearly everywhere in our environment. In water PAH occur either in solution or adsorbed on to particulate material. As certain PAH are known to be highly carcinogenic, six easily detectable PAH were specified, in order to be used as indicators for the large group of PAH. The structural formulae of these six compounds are given in **Table 1**. In 1971 the World Health Organization (WHO) set the maximum acceptable level of the sum of these six PAH in potable waters at 200 ng L^{-1}. This standard was adopted by the European Community, who fixed the maximum admissible concentration for the sum of the six reference substances in drinking water to 0.2 μg L^{-1}, calculated as carbon.

Thin-layer chromatography (TLC) is one analytical method for the determination of these PAH, in addition to other chromatographic techniques. For standard chromatograms, standard solutions containing fluoranthene with five times the level of the other PAH are usually used. This is based on the fact that fluoranthene is present at approximately five times the level of the other PAH in the majority of water samples. A TLC method using development in two dimensions with two different solvent systems has been known for many years. After separation, the PAH are visualized by irradiation with ultraviolet light, and normally the concentration range of the PAH is visually estimated by comparison with standard chromatograms. In addition to this old semiquantitative TLC method, some modern and more efficient TLC methods have been devised.

The improvement of plate material leading to high performance thin-layer chromatography (HPTLC) allowed the development of efficient procedures for qualitative and quantitative analysis of PAH. A big advantage of the HPTLC methods is the possibility of applying up to 18 samples on to one plate, whereas with the two-dimensional method only one sample could be analysed per plate.

Before the PAH can be separated by TLC, they must be extracted from water samples using, for example, cyclohexane. For a sample volume of 1 L 25 mL of solvent is suitable; larger volumes may be convenient in some cases, e.g. when emulsions form. The solvent extract is concentrated to a small volume and, if necessary, materials interfering in the analysis are removed from the extract using column chromatography with alumina or silica gel. The methods described in the following paragraphs can also be applied to extracts of food, soil, smoke and